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A REVIEW ON LIQUID CHROMATOGRAPHY-MASS SPECTROSCOPY AND IT'S APPLICATIONS

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ABSTRACT

The Liquid Chromatography-Mass Spectrometry (LC-MS) is an influential analytical technique with very high sensitivity and specificity. LC-MS is the combination of Liquid Chromatography (LC) and Mass Spectrometry (MS). With the Liquid Chromatography (LC) the separation of components can be done, and then the sample eluents from LC are transferred into Mass Spectrometry (MS) where the detection, identification, and determination of masses of components can be done in the presence of other elements. LC-MS is used in the resolution, of pharmaceutical drug substances, intermediates and its related compounds for the quantitative and qualitative purpose. LC-MS is used most significantly in bio-equivalence, in-vitro dissolution bioavailability and metabolite studies. Also, LC-MS is used in fundamental research, agrochemical, forensic laboratories and food industries. In this article principle of LC-MS, instrumentation and its applications are briefly discussed.

KEYWORDS: Liquid chromatography (LC); Mass spectrometry (MS); Liquid Chromatography-Mass Spectrometry (LC-MS), bio-equivalence, in-vitro dissolution, bioavailability.

INTRODUCTION

The development of combinations of liquid chromatography along mass spectrometry in an online trend has arrived at a level where such combinations become part of routine procedures for the analysis of a broad variety of samples. High-performance liquid chromatography connected along mass spectrometry (LC/MS) is a major permissive technology for the identification and description of organic molecules, contributing the

examining analyst along sole dominant of analytical techniques of today's times. Mass spectrometry has a fast and serious result as an outcome of the greater responsiveness and the resource of structural information for the analysis of organic compounds ranging from low molecular weight drug molecules to large molecular weight biopolymers. LC-MS technique, using LC as a separation system and MS as a detection system, finally achieves the spectrum. Liquid chromatography-mass spectrometry (LC-MS) is now becoming a routine technique with the development of electrospray ionization(ESI) providing a simple and robust interface. It is an effective identification method that incorporates the resolving power of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography (LC) separates the sample components and then introduces them to the mass spectrometer (MS). The LC-MS after long-term development that has introduced seven major interfacing techniques is finally suitable for application in the field of analytical toxicology.

Liquid Chromatography

The Chromatography divides the combined compounds employing the dissimilarities of the distribution coefficient among both phases (mobile and stationary phase). Analogous to the condition of the immovable aspect, chromatography possibly splits into, liquid chromatography, gas chromatography, and supercritical fluid chromatography, although, corresponding to the proportional system of the fixed state, chromatography may be detached into column chromatography, and thin layer chromatography, paper chromatography. The uttermost ordinarily employed Liquid Chromatography technique is column chromatography whichever deference fluid like a mobile phase. High-performance liquid chromatography (HPLC) is changed on the conventional liquid column chromatography. The utilization of Liquid chromatograph is branched into two sections. One is quantitative or qualitative in consideration of a particular configuration. Qualitative measurement is handled according to the consistency between the sample and the target component in the peak time.[1] Quantitative measurement is handled according to the standard curve generated after standards are injected at different concentration levels. The other one is a fingerprint which invokes to the impression that, subsequently the fingerprint fragment obsolete biased off in any form, [we] can obtain chromatogram or spectrogram marked chemical nature by utilizing certain techniques of identification. Liquid Chromatography enjoys a considerable benefit on the efficiency of dividing convoluted samples, remarkably it is the ultimate choice when applied to separate mixtures, but not suitable to obtain structural information of the material.[2] Qualitative investigation completed by the difference amid the peak points of obscure admixtures and the

principle values are not present for monitoring of unknown compounds. LC can be applied to the separation of any mixture that is soluble in a liquid phase.

Mass Spectrophotometry

Mass spectrometry (MS) is employed to analyze combinatorial libraries[3] sequence biomolecules[4], and help explore single cells[5] or objects from outer space. Structure elucidation of unknown substances, environmental and forensic analytes, quality control of drugs, foods, and polymers: they all depend on a great extent on mass spectrometry.[6,7] Today, "mass spectrometry is interwoven with biology to the extent that basic considerations of proteomics research are dealt with in an MS journal".[8]

Though the principles of a modern analytical mass-spectrometer are easily understood this is not necessarily true for the apparatus. A mass spectrometer especially a multi-sector instrument is one of the most complex electronic and mechanical devices one encounters as a chemist. Therefore this means high costs at purchase and maintenance besides a specialized training for the operator. Mass spectrometry is mostly employed in the area of TCM research because of its greater perception and capacity of providing information including relative molecular mass and structural characteristics. MS completes the Qualitation using molecular mass and relevant structural details and achieves quantitation by the relationships of the peak and compound content which the peak represented. Atmospheric pressure ionization (API) of MS has electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI).[9] For different varieties of compounds, Electrospray ionization having larger resp. Correlated with ESI, APCI is appropriate for the lesser polar combinations and the examination of volatile compounds. Rely on the dissimilarities between mass investigators generally employed Mass Spectrometry end with quadrupole mass spectrum (Q-MS), time-of-flight mass spectrum (TOF-MS), and ion trap mass spectrometry (IT-MS).[10,12] Tandem mass spectrometry specify to two or more MS working together. The most commonly used tandem mass spectrometry is triple-quadrupole mass spectrometry (QQQ-MS). In order to use quadrupole to conduct multistage mass spectrometry, three quadrupoles are sequentially placed, which is triple quadrupole.[13] Another type of tandem mass spectrometry, like quadrupole-time-of-flight mass spectrometry (Q-TOF-MS) and, also consists of a variety of quality analyzer series.[14,15] Ion trap time series can attain large number of MS scans eventually at times, so this study sort by type of IT-MS like tandem mass spectrometer. Tandem mass spectrometry can fragments of molecular ions generated by first-stage MS,

according to which we can infer the relationship between child and parent, obtain structural information of the molecule and then suggest the structure of the compound, and conduct the qualitation analysis for known and unknown compounds more accurately.

A typical mass spectrometry instrument has three components as shown in **fig. 1**.

1. Ion source
2. Analyzer
3. Detector: The detector records the current produced when an ion passes by or hits a surface. Several types of sensor are used like electron multiplier, Faraday cups and ion to photon detectors.

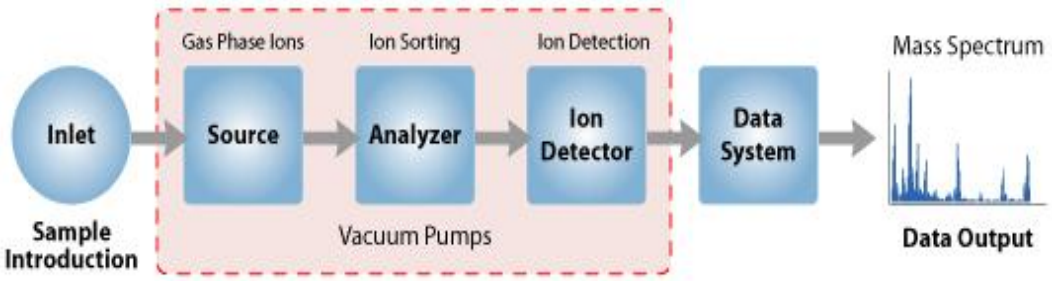

Fig. 1: Mass spectrometry schematic diagram.

Liquid Chromatography-Mass Spectrophotometry

The Liquid Chromatography-Mass Spectrometry (LC-MS) is the highly advanced analytical approach that is in a union of Liquid Chromatography (LC) and Mass Spectrometry (MS). HPLC (LC) divides the consolidation of the blend by moving through a chromatographic column. Mainly, the split mixture can't be easily recognized LC separately. Mass Spectrometry is additionally employed for investigation of mysterious compounds, identified compounds and to exemplify the structural arrangement. Mass spectrometry individually not suitable for analyzing blends on account of collective spectrum blend is, in reality, a mixture of coinciding spectra from divided single components. An attachment is used to transfer the liquid eluents from LC to MS. LC-MS is more significantly used in invite dissolution, bioavailability, bioequivalence and pharmaco-dynamics studies.[16] Preparative LC-MS machines may be used for the rapid mass-directed cleansing of specific substances from such mixtures that are important in basic research, pharmaceutical, agrochemical, food and other industries.[17]

Liquid Chromatography-Mass Spectrometry (LC-MS) or High-Pressure Liquid Chromatography-Mass Spectrometry (HPLC-MS) is an analytical technique that coupled high-resolution chromatographic separation with sensitive and specific mass spectrum detection. This includes Capillary Electrochromatography (CEC)-MS, Capillary Electrophoresis (CE)-MS, High-Performance Liquid Chromatography (HPLC)-MS, and. The blend of Gas Chromatography and Mass Spectrometry (MS) was first reported in 1958 and made available commercially in 1967.[18] When the LC and MS work together, they can carry out multistage MS to speculate the structure of the compound, thus finishing qualitative and quantitative analysis more accurately.[19]

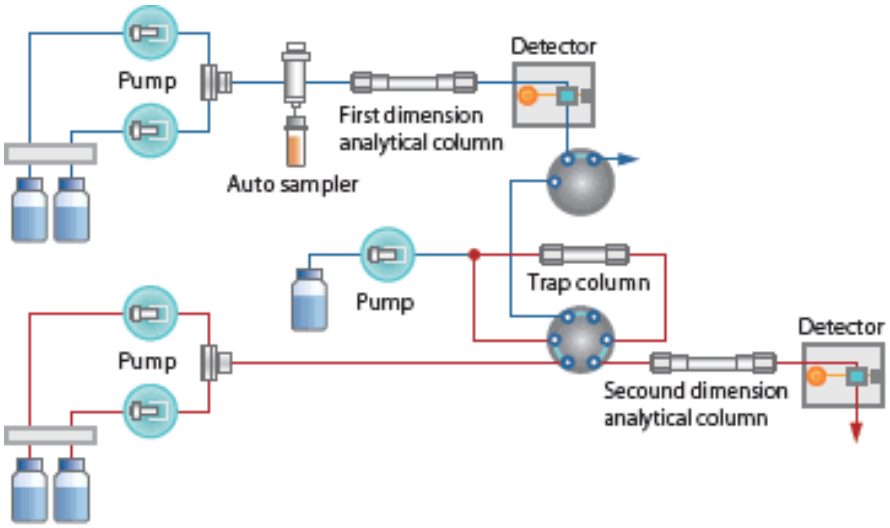

Fig. 2: LC-MS.

Basic Principles of Liquid Chromatography

The common LC-MS system is blended of HPLC and MS employing link (ionization source) (**Fig. 3**). LC divides the specimen, and the both divided sample varieties are sprinkled in atmospheric pressure ion origin, where they are changed within ions in the gas form. The mass analyzer is suddenly employed to separate ions as per to the mass upon charge proportion and analyzer tally the ions coming from the mass analyzer and in addition to propagating the signal created from every ion. Appropriately, mass spectrum (a plot of the ion signal as a function of the mass-to-charge ratio) is created, which is used to determine the elemental or isotopic nature of a sample, the masses of particles and molecules, and to elucidate the chemical structures of molecules.[20]

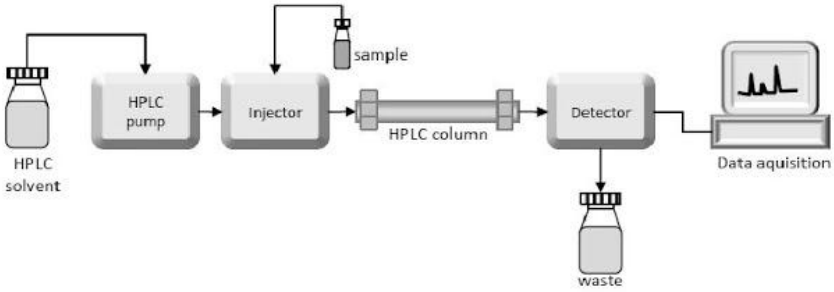

Fig. 3: HPLC-MS.

Present day liquid chromatography generally utilizes microscopic particles packed and operating at relatively high pressure, and is referred to as high-performance liquid chromatography (HPLC); modern LC-MS methods use HPLC instrumentation, substantially exclusively, for a sample. The fundamental principle in HPLC is adsorption. Use of octadecylsilyl (C18) and related organic-modified particles as a stationary phase with pure or pH-adjusted water organic mixtures such as water-acetonitrile and water-methanol are used in techniques termed as reversed phase liquid chromatography (RP-LC). Use of components like silica gel being the stationary phase with pure or blended organic elements are utilized in methods called as normal phase liquid chromatography (NP-LC). RP-LC is uttermost often used as the means to introduce samples into the MS, in LC-MS instrumentation.[21]

Instrumentation of Liquid Chromatography-Mass Spectrometry

The Liquid Chromatography-Mass Spectrometry (LC-MS) is the unification of Liquid Chromatography and Mass Spectrometry that is employed with partition power of HPLC with disclosure ability of Mass Spectrometry (MS). The illustrative section picture of LC-MS is presented in **Fig. 4**. The various sections of LC-MS instrument are described as under.

1. Liquid Chromatography
2. Mass Spectroscopy

Liquid Chromatography (LC)

The Liquid Chromatography may be defined as a high-performance liquid chromatography into whatever partition of different parts of the mixture can be done by utilizing liquid mobile and solid, immovable phase. As per availability, there are various types of chromatography such as affinity liquid chromatography, Chiral separation, Ion-exchange liquid chromatography normal phase liquid chromatography, Reversed-phase chromatography.[22]

By using different packing of columns with the high-efficiency small amount of complex mixture can be separated. The components of HPLC are listed below:

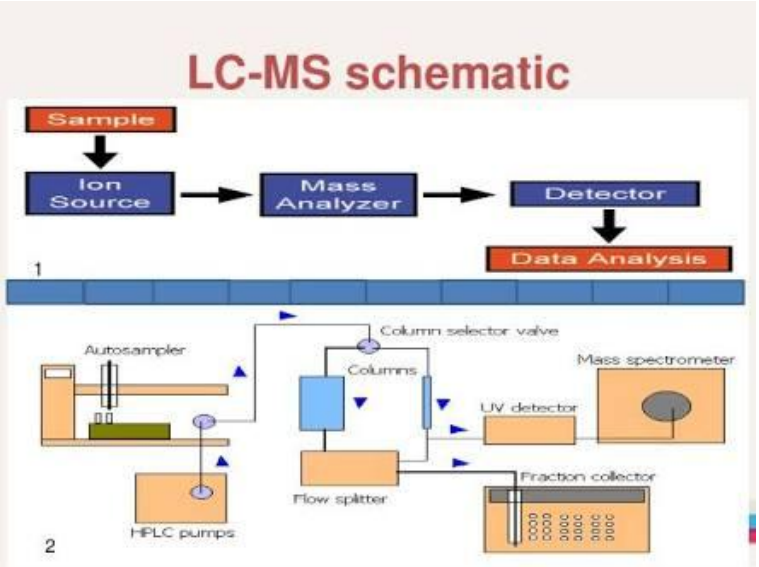

Fig. 4: LCMS schematic diagram.

a. Pump: It contained the element that is stable for other compounds or some blends of watery buffer and organic liquids. It conveys more massive amounts of mobile phase about 10mL/min. Here are three dominant varieties of pumps are, i.e., constant pressure pumps, reciprocating pump, Syringe pumps, and

b. Sample injector mainly employed for introducing sample amount in the chromatographic machine. Usually, sample amount ranging 1µL to 100µL should be presented. The volume of injection can be incremented by using injector loop about 2mL volume. Here are two dominant varieties of injectors employed, i.e., Automatic injectors and Manual injectors. Automatic injectors are comfortable and user-friendly and are more accurate and precise as compare to manual injector.[22]

c. Columns: This part consists of the phase consisting of silica in the form of the blend with carbon chain. Usually, the column having a length of 50mm to 300mm is used. The columns utilized in HPLC consisting of Octadecyl (C18), Octyl (C8), Cyano, Amino, Phenyl packing. The columns are being used by nature of compounds to be separated.[23]

d. Detectors and recorder: The indicators are most prominent part of HPLC. here are various types of sensors employed are Fluorescence detectors, UV-Visible detectors, conductivity detectors, PDA detectors, Refractive index (RI) detectors, and Electrochemical

detector. The signal taken from the sensor can be recorded as peak, and respective data can be stored in the application.

Mass Spectrometry

Mass Spectrometry is that identification technique relies on the calculation of the mass to charge ratio of the analyte underneath the probe.MS can be employed to define the molecular mass and component composition of an analyte including in-depth structural elucidation of the analyte. In LC-MS there are two significant components, ionization origin, and Interfaces. Beneath cataloged are the different elements of Mass spectrometers as listed.

i. Ionization Sources and Interfaces

ii. Mass Analyzers

Ionization/Ion origin and Interfaces

The Liquid chromatography divides mixture of different components which are present in liquid form, usually contains acetonitrile methanol and water. The liquid consisting mixture of various elements is moved into the ion source of the mass spectrophotometer. As ion source is under high vacuum. Because of the variations in the pressure, it is hard to evaporate the liquid globules without loss of the mixture of components. Therefore links are used to solve this difficulty. The different types of interfaces generally employed in the mass spectrometer are listed under.

a. Direct liquid Introduction (DLI): The ionization in Direct Liquid Introduction (DLI) is usually accomplished by evaporating solvent like chemical ionization and reagent gas. The two regular and reverse phase solvent system has been employed. Reverse phase solvents used are water, acetonitrile/ methanol/ water mixture up to 60% water. Conventionally buffer combined with salts are not admissible as there is the possibility of capillaries to stopple when scorched. The operation of Direct Liquid Introduction (DLI) is a mixture of thermal energy and liquid flow ratio. The liquid enters the interface at reduced flow rate only. The analyte ions formed with the help of thermal energy then transferred into ion source through the capillary inlet or pinhole diaphragm.[24]

b. Atmospheric-Pressure Ionization (API): In Atmospheric-pressure ionization (API) consisting of three significant steps, i.e., Ionization, Evaporation, and Nebulisation. There are two major modes of API are Atmospheric-pressure ionization (APCI) Electrospray Ionization (ESI) and. In Atmospheric-pressure ionization (API), when the stream of

compound (solvent) containing a sample is moved through a confined capillary tube and nebulized at the vast chamber, the mist of small droplets are formed. The ionization process takes place, and the ratio of droplets carry an extravagance of positive or negative electric conduct.In the large heating chamber, the drying up of solvent takes to succeed. The water dried up from the small drops to form most diminutive. The concussion took place between the molecules and ions. The resulting ions then passed through the capillary into mass analyzer.[25,26] Electrospray (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization ionization (APPI), DESI and DART are other examples of atmospheric pressure ionization (API) sources.

c. Electrospray Ionization (ESI): The Electrospray Ionization (ESI) is more useful ion origin developed by Fenn and his counterparts. In Electrospray Ionization (ESI) the liquid sample moved through a stain steel capillary tube which is maintained at more substantial positive or negative electric potential about 3-5kV.[27] Due to this, the charged droplets are formed at the capillary tip which is then undergoing vaporization process. The solvent gets evaporated from droplets and undergoes a reduction in size and surface charge increases. The collision takes place until the highly charged drops are changed into gas phase ions. These gas-phase ions pass through the capillary sampling orifice into the low-pressure region of the ion source.[28] In the literature, electrospray is abbreviated to either ESI or ES. Because ES is ambiguous, we prefer to use ESI. The success of ESI started when Fenn *et al.*[29,30] showed that multiply charged ions were obtained from proteins, allowing their molecular weight to be determined with instruments whose mass range is limited to as low as 2000 Th. Later on, its use was extended not only to other polymers and biopolymers but also to the study of small polar molecules.

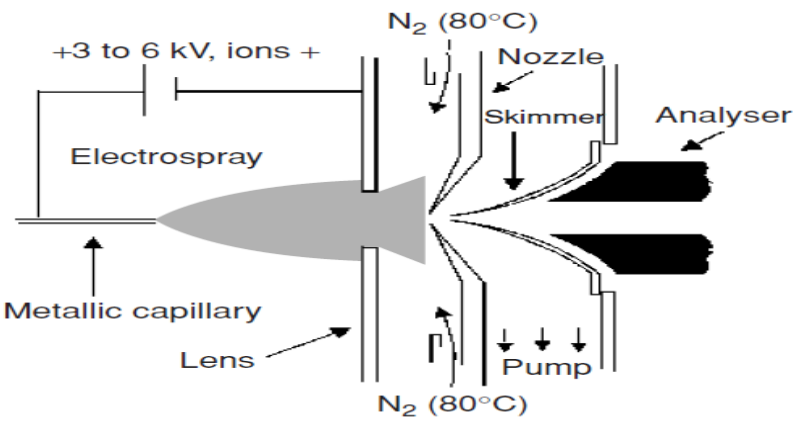

Fig. 5: Diagram of Electrospray Ionization.

The significant benefits of ESI are that the ions are highly charged, the total figure of charges elevated by 1 to 3 for a molecule 1000Da or above-raised 50000Da. This earns an m/z ratio which is always beneath 2000. LC-MS combined with an Electrospray ionization (ESI) is employed to calculate the molecular weight of proteins peptides, sugars, Biological samples, Polymers, nucleotides and organometallics. It is also utilized frequently in Biological research and medical analysis.[31]

d. Atmospheric Pressure Chemical Ionization (APCI): The Atmospheric Pressure Chemical Ionization (APCI) consisted two dominant steps, desolvations/ evaporation of analytes and charged deportation reaction in vapor form to create the vapor form ions. In Atmospheric Pressure Chemical Ionization (APCI) fluid (solvent) consisting sample is nebulized from the confined capillary tube and nebulized into the vast chamber. In colossal heating chamber, the dispersion of solution intervene at atmospheric pressure, and small drops are produced. The ionization happens. Generally, ionization occurs at 250 to 400 °C. The ions then move the charges to molecules by chemical reactions. The resulting ions are transferred from a narrow opening of the mass analyzer. It is mainly used for less polar and non-polar analytes having moderate molecular weights.[32]

e. Thermospray ionization; The principle of the thermospray (TSP), proposed by Blakney and Vestal in 1983, there is solution containing a salt and the sample to be analysed is pumped into a steel capillary, which is heated to high temperature allowing the liquid to heat quickly. The solution passes through a vacuum chamber as a supersonic beam. A fine-droplet spray occurs, containing ions and solvent and sample molecules. The ions in the solution are extracted and accelerated towards the analyser by a repeller and by a lens focusing system. They are desorbed from the droplets carrying one or several solvent molecules or dissolved compounds. It is thus not necessary to vaporize before ionization: ions go directly from the liquid phase to the vapour phase. To improve the ion extraction, the droplets at the outlet of the capillary may be charged by a corona discharge. The droplets remain on their supersonic trip to the outlet where they are pumped out continuously through an opening located in front of the supersonic beam. Large vapour volumes from the solvent are thus avoided. In order to avoid the freezing of the droplets under vacuum, the liquid must be heated during the injection. This heating is programmed by feedback from a thermocouple which measures the beam temperature under vacuum. The charged drops are formed. Due to the dispersal of solvent the drops become smaller. The density of electric charge on the outer side of droplets

increases. The resulting ions are then passed into mass analyser with electrostatic voltage system.[33] The Plasma spray itself will not produce ions but the ions created in thermospray, with the assistance of corona discharge or plasma the number of ions can be elevated. The electric discharge increases the more ionization in the inert molecules. This enhancement of expansion of the ionization of compound. The plasma spray method is more sensitive and it is widely used for analysis in clinical and medicine.[34] The Thermo spray is employed as both liquid injection system including ionization source. Plasma spray is the conversion of thermospray. In Thermo spray the liquid solution is moved through capillary tube that is heated and which causes the dispersal of solvent. The charged drops are formed. Because of evaporation of solvent the drops becomes tinier. The density of electric charge at the outer side of droplets increases. The resulting ions are then passed into mass analyser with electrostatic voltage system.[33]

f. Atmospheric Pressure Chemical Ionization

APCI is an ionization technique which uses gas-phase ion-molecule reactions at atmospheric pressure. It is a method analogous to CI (commonly used in GC–MS) where corona discharges produce primary ions on a solvent spray. APCI is mainly applied to polar and relatively non-polar compounds with moderate molecular weight up to about 1500 Da and generally gives monocharged ions. The principle governing an APCI source is shown in Figure. The analyte in solution from a direct inlet probe or a liquid chromatography eluate at a flow rate between 0.2 and 2mlmin−1, is directly introduced into a pneumatic nebulizer where it is converted into a thin fog by a high-speed nitrogen beam. The gas flow then displaces droplets through a heated quartz tube called a desolvation/vaporization chamber. The heat transferred to the spray droplets allows the vaporization of the mobile phase and the sample in the gas flow. The temperature of this chamber is controlled, which makes the vaporization conditions independent of the stream and from the nature of the mobile phase. The hot gas (120◦C) and the compounds leave this tube. After desolvation, they are carried along a corona discharge electrode where ionization occurs. The ionization processes in APCI are equivalent to the processes that take place in CI, but all of these happen under atmospheric pressure.

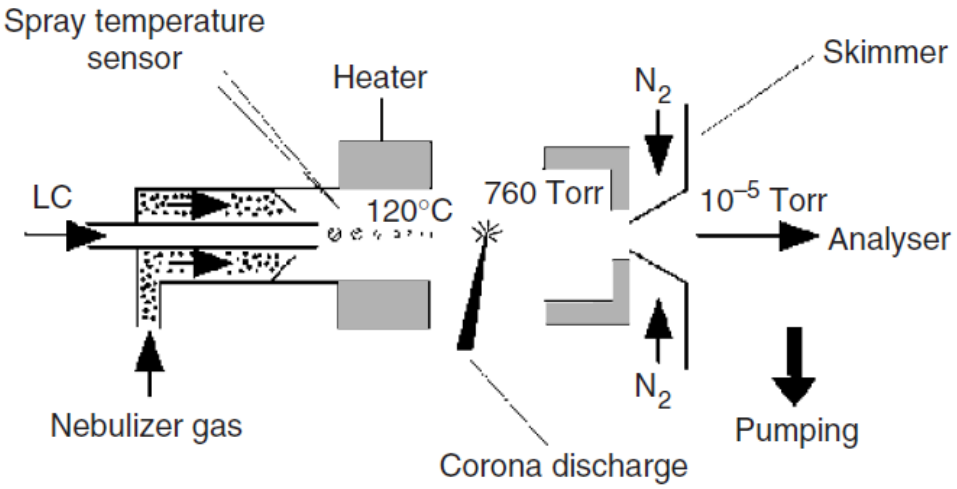

Fig. 6: Diagram of APCI.

e. Plasma spray Ionization (TSPI)

The Plasma spray itself shall not make ions but the ions formed in thermospray, with the help of corona discharge or plasma the number of ions can be raised. The electric release induces the more ionization in the unbiased molecules. This increment increases the ionization of molecule. The plasma spray method is more sensitive, and it is widely used for identification in clinical and medicine.[35]

f. Atmospheric pressure photo Ionization (APPI): In Atmospheric pressure photo Ionization (APPI), photons are employed to excite and ionize the fragments. Atmospheric pressure photo Ionization (APPI) include chiefly two steps which are excitation and ionization of analyte from eluent. Such as atmospheric pressure chemical ionization (APCI) in Atmospheric pressure photo Ionization (APPI) the eluent from LC vaporizes in the gaseous phase. The APPI uses Kr lamp to produce photons. Kr lamp creates high energy photons which are employed for excitation and ionization of molecules. The range of energy is elected to minimize the ionization of analytes. The ionized analytes are then moved into capillary orifice into the mass analyzer (m/z). This technique is essential for non-polar analytes which highly challenging to ionize with Atmospheric Pressure Chemical Ionization (APCI), Electrospray Ionization (ESI).[36]

g. Particle Beam Ionization: The Browner and his colleagues have developed particle beam interface to separate the solvent from solute with minimum loss of solutes. The nebulization and evaporation process is like Thermo spray (TSP), Atmospheric pressure chemical ionization (APCI), Electrospray ionization (ESI).[37] In this liquid separated from HPLC or

LC, the eluent is passed through the narrow tube. The fluid is injected with helium gas, due to this, the spray of liquid droplets are formed with high velocity. The liquid drops from nebulizer pass through the heating chamber, where the solvent begins to evaporate and liquid droplets become smaller and smaller. The spray of liquid droplets exits through the heating chamber as a particle beam. Then this beam passes through ionization chamber similarly like Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI).

h. Continuous Flow Fast Atom Bombardment (FAB): The fast atom bombardment is highly sensitive, simple method of interface. In FAB liquid goal is collided by fast atoms like Argon(Ar) or xenon. The sample is mixed in glycerol and spread on thin layer metal plate /probe. Then this probe is inserted into the mass spectrometer, and a beam of fast-moving atoms bombards on the examination and ionize the samples which then pass into the mass analyzer (m/z). FAB is used for huge and thermally unstable molecules. It applied for surfactants and proteins.[38,39]

Working of LC-MS

LC/MS is typically applied to the analysis of multiple component mixtures. Liquid chromatography resolves each component and determined by atmospheric pressure ionization mass spectrometry. Atmospheric pressure ionization mass spectrometry (API-MS) operates by ionizing sample molecules in the liquid chromatographic mobile phase to produce a beam of gaseous ions. Ionization is carried out at atmospheric pressure for dual reasons; firstly, heat transfer more efficiently enhances solvent evaporation and, secondly, high electric fields do not conclude in strong electrical discharges that occur at retarded pressure. API-MS includes atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI). The two methods are same in that they ionize sample molecules to yield gaseous ions but distinct in return to sample chemistry. ESI is more suitable to the analysis of ionic, polar compounds, while APCI works with relatively nonpolar compounds. Once gaseous ions are formed, they are electrostatically extracted into a heated capillary positioned orthogonally to the spray. Ions from the heated capillary are transmitted to the quadrupole mass analyzer using an innovative ion-optic design for high ion transmission conclude in high responsiveness. The configuration integrates a quadrupole array with an octapole ion bridge to properly focus ions from the ion source in the quadrupole mass analyzer Ions from the heated capillary are transmitted to the quadrupole mass analyzer using an accelerating voltage resulting in a mass spectrum often dominated by the pseudo- molecular ion with little fragmentation. When the

energy acquired by the molecule surpass the ionization potential, the excess energy is distributed inside the molecule and when the power equals the dissociation energy for a particular chemical bond fragmentation occurs. Fragmentation provides a great deal of information about the structure of the molecule and is controlled by applying an accelerating voltage after the heated capillary. This technique of 'in source fragmentation' is particularly useful in structural elucidation and compound verification analysis.

Mass Analyzers

Once the gas-phase ions have been produced, they need to be separated according to their masses, which must be determined. The physical property of ions that is measured by a mass analyzer is their mass-to-charge ratio (*m/z*) rather than their mass alone. Therefore, it should be mentioned that for multiply charged ions the apparent *m/z* values are fractional parts of their actual mass. As there are a great variety of sources, several types of mass analyzers have been developed. Indeed, the separation of ions according to their mass-to-charge ratio can be based on different principles. All mass analyzers use static or dynamic electric and magnetic fields that can be alone or combined. Most of the essential differences between the various common types of mass analyzer lie in the manner in which such areas are used to achieve separation. Each mass analyzer has its advantages and limitations. Analysers can be divided into two broad classes from many properties. Scanning analyzers transmit the ions of different masses successively along a time scale. They are either magnetic sector instruments with a flight tube in the magnetic field, allowing only the ions of a described mass-to-charge ratio to pass through at a given time, or quadrupole instruments. However, other analyzers allow the simultaneous transmission of all ions, such as the dispersive magnetic analyzer, the TOF mass analyzer and the trapped-ion mass analyzers that correspond to the ion traps, the ion cyclotron resonance or the orbitrap instruments. Analysers can be grouped from other properties, for example, ion beam versus ion trapping types, continuous versus pulsed analysis, low versus high kinetic energies.

Quadrupole Analyzers

The quadrupole analyzer is a device which uses the stability of the trajectories in oscillating electric fields to separate ions as per their *m/z* ratios. The 2D or 3D ion traps are based on the same principle. Quadrupole analyzers[40] are made up of four rods of circular or, ideally, hyperbolic section. The rods must be perfectly parallel.

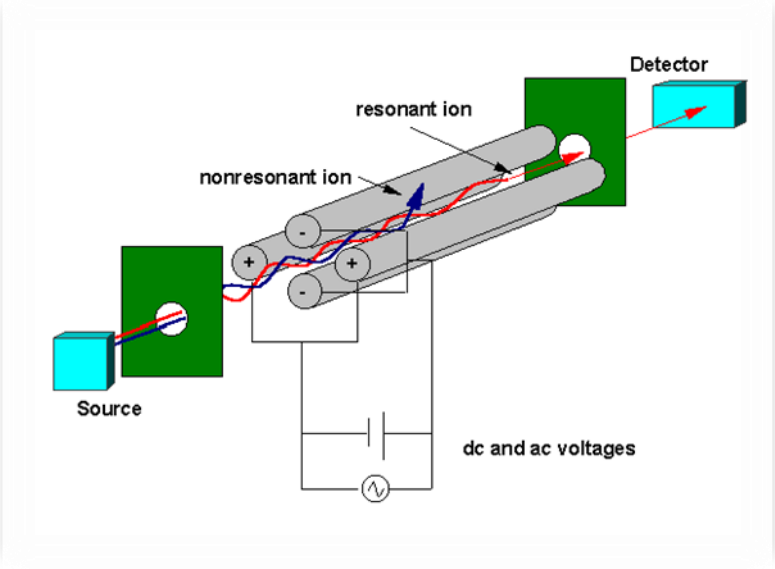

Fig. 7: Schematic Diagram of quadrupole mass analyzer.

The principle of the quadrupole was described by Paul and Steinwege[41], at Bonn University, in 1953. They started from research work on the intense focusing of ions carried out in 1951 in Athens by the electrical engineer Christophilos. The quadrupoles have since been developed into commercially available instruments by the work of Shoulders,[42] and Story.

It is the most useful and generally used mass Analyser. It consists of two plain of parallel rods which are located between an ion source and a detector. The mass Analyser, i.e., separation of ion according to their m/z in either time or space.

The linear Quadrupole mass Analyzer consists of four hyperbolic or cylindrical rods that are placed parallel in a radial array. Opposite rods are charged a +ve or –ve direct current(DC) potential at which an oscillating radio frequency alternating current (RF) voltage is superimposed.[43] The combination of DC and RF applied to the rods, trajectories of the ions of one particular m/z are stable these ions are transmitted towards the detector. On the other hand ions of unstable m/z are discharged on the rods.

The ions introduced into Quadrupole by mean of low accelerating potential. The ions are oscillating in a plane perpendicular to the rod length as they trend through the Quadruple filter.

Ions were carrying m/z consequently be traveled towards detector by applying DC and RF voltage at the constant ratio. The resolution depends on the rate of DC and RF potentials.

Generally, the Quadrupole is operated at <4000 m/z and scan speed up to 1000m/z passes. The unit mass resolution means that mass accurately is seldom better than 0.1 m/z.[44]

Time-of-Flight

In a time-of-flight (TOF) mass spectrometer, the ions generated in the ion source are accelerated through a known potential and travel through a flight tube to reach the ion detector. The ion arrival time at the detector is measured, and it is proportionally related to the *m/z* values of ions. It takes a larger time for heavy ions to reach the detector, while light ions arrive at the detector earlier.[M. Guilhaus et al]. In spite of the linearity of the technique, the high performance of TOF rely on the corrections regarding flight time differences of the ions with the same *m/z* values that are caused by initial kinetic energy spread, initial angular spread, and initial position of ion formation spread.A well-established method for velocity differences is to use the reflection to compensate for the kinetic energy differences.[45] In this technique, more energetic ions permeate deeper in the reflectron field so that these ions will have a larger flight path and take place at the detector at the equal time as ions with smaller kinetic energy. Other instrument renovation have also been made to achieve high performance capability. The main data acquisition method in TOF is time to- digital converter. It is a time counting device and provides high time resolution. In contrast to scanning mass analyzers (magnetic sector, quadrupole and ion trap), all ions in TOF are almost progressively detected, further improvement of sensitivity. One of the exclusive aspects of TOF is the need of pulsing ions for measurements. Thus TOF is best used with ion sources forming ions in pulses. Basically, MALDI is ideal for the combination with TOF. In fact, MALDI-TOF is one of the most widely used systems for analysis of large biomolecules. For a continuous ion source, the ions can be stored for a short period of time and pulled out for analysis. In the case of ESI, orthogonal injection provides efficient injection of ions from ESI source to TOF. Other important characteristics of TOF also include high mass range (over 100,000 Da), high resolution (over 10,000), and good mass accuracy for accurate mass measurements (less than 5ppm). These features make TOF attractive for high-resolution LC/MS and LC/MS/MS applications, especially with the combination of quadrupole and TOF system.

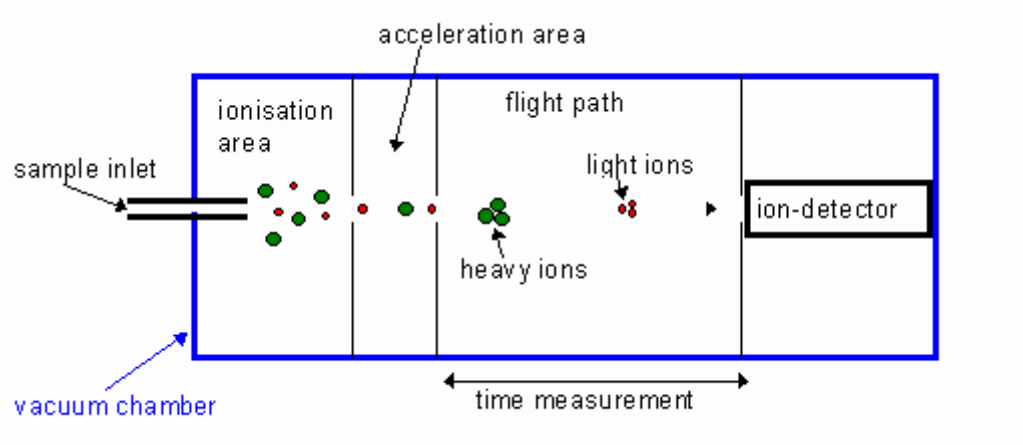

Fig. 8: Schematic diagram of the time of flight analyzer.

Ion Trap Analyzer

The quadrupole ion trap is a three-dimensional (3-D) analog of the linear quadrupole.[46] It consists of two end-cap electrodes with hyperbolic cross-sections and one ring electrode located between the end caps (Figure). The RF voltage is tested to the ring electrode, and the ground potential is usually operated on the end caps. A rotationally symmetric electric quadrupole field is produced in the device to trap ions with stable motions explained by Mathieu equation. The mass spectra are retrieved by raising RF voltage to instigate the ions to become unstable and be expelled from the trap through a hole in the end-cap electrode. In this mass-selective instability scan mode, ions of increasing mass-to-charge ratios are ejected and identified as the RF voltage is increased. The trapped ions possess oscillation frequencies. The stable movement of ions in the trap is assisted by the presence of a helium buffer gas (1 mtorr) to remove kinetic energies from ions by collisions. When a supplementary AC potential, corresponding to the frequency of a specific *m/z* ion, is applied to the end-cap electrode, ions are resonantly ejected from the trap. This method of resonance ejection is used to extend the mass-to-charge ratio of the ion trap effectively. Some other characteristic features of a 3-D ion trap include high sensitivity, high resolution with slow scan rate, and multiple-stage MS capability (see the section on tandem MS). Also, it is inexpensive and little in size. As a result, a 3-D ion trap is widely used in LC/MS/MS, LC/MS applications. One of the innate limitations to a 3-D ion trap is the ion storage capacity because of the relative little volume in the trap. The space-charge effect can be symbolic when the ion numbers arrive above 106 ions, mass accuracy, impacting mass resolution, sensitivity, and dynamic range. To overthrown this limitation, a 2-D linear ion trap has been designed to improve the performance of 3-D ion trap.[47] Its quadrupole structure has a

hyperbolic rod profile, same as the conventional quadrupole rod. In one of the configurations in a 2-D linear ion trap, the quadrupole rod is trimmed into three axial sections (front section, center section, back section). Suitably DC and RF potentials are applied to the three parts to contain ions along the axis in the middle part of the device. The ion detection is achieved by ejecting ions out of the trap through a hole in the center section. Dual ion detectors along the center section have been used to improve the sensitivity. The advantages of a 2-D linear ion trap over a 3-D ion trap are the increased ion storage capacity(at least ten times more than 3-D ion trap) and higher trapping abilities, leading to better responsiveness and a higher dynamic range. The use of 2-D linear ion trap is getting popularity in LC/MS and LC/MS/MS function, either as a stand-alone instrument or in combination with other mass analyzers.

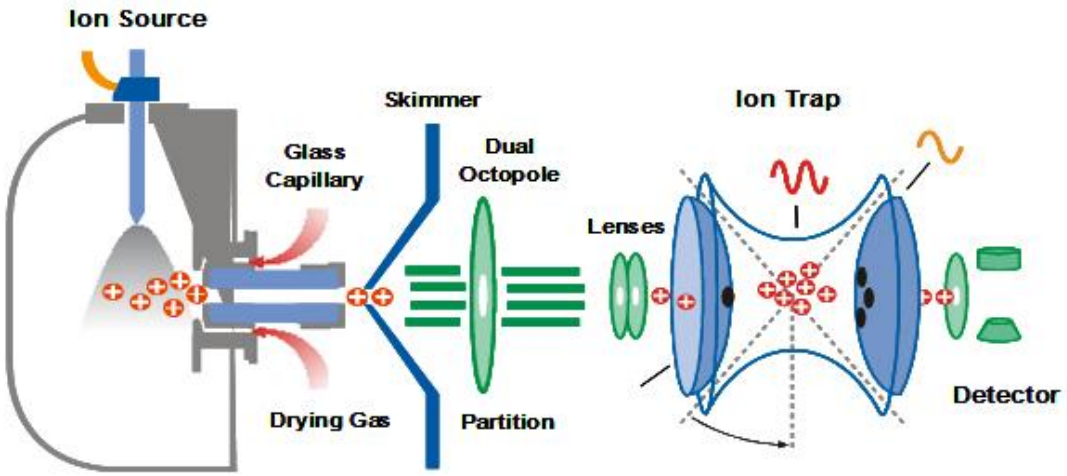

Fig. 9: Ion trap analyzer.

Detectors

A detector is an essential tool of mass spectrometer that produces the current that is proportional to the number of ions strike it. Once the ions are formed passed from analyzer, they have to be detected and transformed into a signal. Below listed are the type of detectors commonly used.

The ions pass through the mass analyzer and are then detected and transformed into a usable signal by a detector. Detectors can generate from the incident ions an electric current that is proportional to their abundance. Sensors used in mass spectrometry were reviewed in 2005.[48] The most common types of ion detectors are described below. Their specifications and their features are also discussed.

Several types of detectors presently exist. The choice of indicator relies on the design of the instrument and the analytical applications that will be performed. A variety of approaches are used to detections. However, detection of ions is always based on their charge, their mass or their velocity. Some detectors (Faraday cup) are based on the measurement of direct charge current that is produced when an ion hits a surface and is neutralized.

Photographic Plate

The first mass spectrometers used photographic plates located behind the analyzer as detectors. Ions sharing the same *m/z* ratio all reach the plate at the same place, and the position of the spots grant the determination of their *m/z* values after calibration. The darkness of the spots gives an approximate value of their relative abundance. This detector, which allows simultaneous detection over a vast *m/z* range, has been used for many years but is obsolete today.

Array Detector

An Array detector is the collection of point collectors placed in the plane. The ions are reach at a point or across the plane in an array detector. The ions having mass to charge (m/z) values are divided and are recorded along plane using point ion collector. Spatially differentiated ions with the mass range are detected simultaneously at the same time in array detector.[49,50]

Comparison Between LC, MS, and LC-MS

Liquid Chromatography (LC)	Mass Spectrometry (MS)	LC-MS
LC is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent by using liquid mobile phase and solid stationary phase.	Mass spectrometry is an instrumental technique in which sample is converted to rapidly moving positive ions by electron bombardment and charged particles are separated according to their masses.	LC-MS is the hyphenated analytical technique which is a combination of Liquid Chromatography (LC) and Mass Spectrometry (MS) which is used with separation power of HPLC with detection power of Mass Spectrometry (MS). Separation and quantitation of components can be done.
In LC, Substances will move with the mobile phase at the different rate depending upon their partition or adsorption.	In MS, the components can be converted into gas phase ions and separate the ions in time or space according to mass from charging ratios. Measures the number of ions of every mass to charge rate.	In LC-MS, the separated components from LC can be transferred into the mass spectrometer with the interfaces. Separation and determination of relative atomic masses or molecular masses can be performed simultaneously.
This technique is used for chemistry and biochemistry research analyzing complex mixtures.	Explanation of the structure of the organic and biological molecules.	Used in Pharmacokinetics, Bioavailability and bioequivalence studies
Used to quantify and separation of drug substances and drug products.	Determination of mole- cular mass of peptides, proteins etc.	Used in metabolite studies, forensic studies.
It is used in pharmaceutical, agrochemical and pesticide industries.	Monitoring gases in patients breathe during surgery.	For determination of assays of drug substances.

Applications

Application of LC/ESI-MS in In forensic sciences

LC-MS is used for toxicity, in drug analysis and also in trace analysis. By using the small amount of sample, the toxins in a different material can be determined with LC-MS. Any toxic metabolites in food or beverages can be determined by using LC-MS. E.g. Identification of detergent added into orange juice can be determined by analysing by the juice and detergent sample. The standard surfactant alkyl diphenyl ether sulphonic acid is used. Both juice and detergent samples are analysed in same chromatographic conditions. The mass chromatograms and mass spectra obtained from the juice and detergent samples are identical with the reference spectra of standard surfactant (alkyl diphenyl ethersulphonic acid).[51]

Determination and Detection of Impurities

LC-MS is employed in pharmaceutical analytical development mainly for searching products for impurities and identifying those that are detected. The technique has brought two dominant advances: the search for impurities is more inclusive, and many impurities can be detected without the need for time utilizing preparative chromatography (on-line LC–NMR is not yet genuine or cost-effective). An identification limit of a few hundred ppm is acceptable in order to comply with the requirement that all impurities above 0.1% were identified. LC-MS would rarely be required for the routine assay of impurities at these levels.

Environmental Applications

Environmental analysis is a very important application area of LC-MS mainly related to the study about the occurrence and fate of organic micropollutants in wastewater, sludge, natural waters, drinking water, sediments, soil, and aquatic biota. The term “organic micropollutants” is meant to include any organic contaminant – pesticides, pharmaceuticals, personal care products, industrial chemicals, hormones, flame-retardants, plasticizers, and others – which enters the environment during its production, consumption, and disposal at ppm or lower level. Among all the known organic contaminants, pesticides are the most relevant and investigated since approximately 900 approved active ingredients, belonging to more than 100 different classes, are still being used worldwide. The same applies to legal and illegal drugs: at present, about 4000 pharmaceuticals are in use in the European Union (EU).[52] Growing concern and awareness about the potential toxicity to living organisms or/and eco-toxicity of these contaminants is the driving force for developing fast and sensitive

multicomponent methods in order to expand monitoring strategies and to investigate the micropollutant fate in the treatment processes and environment.

Applications in forensic science

Toxicology

Drugs of abuse: Traditionally, laboratories use GC–MS for the confirmation of illicit drug use. However, this is a time consuming and labor-intensive process, particularly as sample preparation; that is, solid-phase extraction (SPE) and derivatization are generally necessary. LC-MS may well be the resolution to the analysis and quantification problems often encountered by analytical toxicologists because it permits the confirmation analysis of polar or non-volatile compounds without the need for derivatization. In comparison to single quadrupole methods, tandem mass spectrometry (MS-MS) offers superior sensitivity and selectivity for the species of interest. These instruments, when operated in multiple reactions monitoring (MRM) mode, enable the quantitative identification of low levels of compounds in biological matrices, often with much smaller sample preparation and identification times. in present time, stable isotopically labelled analogues of countless drugs of interest are easily available. These prove to be priceless tools for the purpose of internal standardization and quantification. Additionally, because these standards have almost identical physicochemical properties to the unlabeled drug, they will compensate for any effects of reduced sample preparation or ion suppression produced by the matrix.[53]

Application of LC-MS in Doping Test

The LC/ESI-MS with positive mode can be used for detection in the urine of 4-Methyl-2 hexane amine doping agent. The urine samples are analysed with the addition of internal standard of Tuaminoheptane. The suspected primary amine 4-methyl-2-hexaneamine, an analog contained in the dietary supplement, to be the unknown compound. The standard used is 4-methyl-2 hexaneamine which reveal two uncertain peaks at RT 3.43min and 3.78min. which are same with those of unknown compound.[54]

Pharmacokinetics

MS is used in the study of metabolism, absorption, and excretion of drugs. Bioanalytical methods are used for quantitative and structural elucidation of drugs and its metabolites in the biological samples (plasma, urine, saliva, serum etc.).

In Bioavailability and Bioequivalence study

Comparative bioequivalence studies in which quantitative determination of drugs or metabolites is measured in a biological matrix, pharmacodynamics, clinical trials and In-vitro dissolution tests.

In the determination of molecular weights

LC-MS is employed for determination of molecular weights of known and unknown compounds. It gives the information about molecular weight, structure, identification, the number of sample components. LC-MS is used for determination of molecular masses of proteins, nucleic acids, polymers and peptides.

In the determination of Assay of drug and intermediates

LC-MS is used in pharmaceutical industry for determination of assay of drug substances, drug products, intermediates and their related compounds.

In Agrochemical and pesticides industry

It is used in determination of different components present in the fertilizers and pesticides.

Molecular Pharmacognosy

LCMS determines the contents and categories of different groups of cultured plant cells and selects the pair of groups with the biggest different content of ingredient for the study ingredient difference phenotypic cloning.

Quantitative Bioanalysis of various Biological Samples

LC-MS/MS methodology includes sample preparation, separation of components and MS/MS detection and applications in several areas such as quantification of biogenic amines, the pharmacokinetics of immunosuppressants and doping control. Advancement including automation in the LC-MS/MS instrumentations along with parallel sample processing, column switching, and usage of more effective supports for SPE, which drives the trend towards less sample clean-up times and total run times–high-throughput methodology in today's quantitative bioanalysis area. Newly introduced techniques such as ultra-performance liquid chromatography with small particles (sub-2μm) and monolithic chromatography offer improvements in speed, resolution and sensitivity compared to conventional chromatographic techniques.

Automated Immunoassay in Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) of certain drugs with a narrow therapeutic index helps in improving patient outcome. The need for accurate, precise, and standardized measurement of drugs poses a major challenge for clinical laboratories and the diagnostics industry. Different techniques had developed in the past to meet these requirements. Nowadays liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based methods and immunoassays seem to be the most widespread approaches in clinical laboratories. Mass spectrometry-based assays can be analytically sensitive, specific and capable of measuring several compounds in a single process. This is a cost-effective approach to examining patients receiving multidrug therapy (e.g., antibacterial therapy for Tuberculosis patients). The selectivity provided by successive mass filtrations is an added advantage of tandem mass spectrometry over immunoassays, as is shown for immunosuppressant drugs.

Clinical chemistry and toxicology

For certain clinical chemistry and toxicology analytes, liquid chromatography (LC) paired with tandem mass spectrometry (MS/MS) offers eloquent advantages over traditional testing by immunoassays. The tested analytes include oestradiol, testosterone, thyroid hormones, immunosuppressants, vitamin D, steroids for newborn screening programs, and clinical and forensic toxicology. While immunoassays are commonly employed in the clinical laboratory, the analytical responsiveness and specificity are lower for many of the analytes identified in routine clinical laboratories. Moreover, LC-MS/MS can be multiplexed for high testing throughput and multiple analyte detections. The application of LC-MS/MS in clinical chemistry and toxicology studies shall improve and the advantages become well known. There are few immunoassays for therapeutic drugs that can cause toxicity if not used properly. The goal of an untargeted analysis is to determine as many of the drugs that are of clinical or forensic importance as possible, irrespective of the availability of an immunoassay. Urine is usually the preferred sample, but serum and blood are also few important sample types.

CONCLUSION

The LC-MS is a hyphenated technique used in combination with separation power of HPLC with detection power of Mass spectrometry. It is mostly used in pharmaceutical, chemical, food, agrochemical industries, environmental and forensic applications. LC-MS is employed

for qualitative and quantitative determination of drug substances and biological samples. Also, it is generally used in drug research and quality control.

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